Bent out of Shape: RNA Unwinding by the DEAD-Box Helicase Vasa

Patrick Linder[1,*] and Paul Lasko[2]
[1]Department of Microbiology and Molecular Medicine, Centre Médical Universitaire, University of Geneva, CH-1211 Genève 4, Switzerland
[2]Department of Biology, McGill University, Montréal, QC H3A 1B1, Canada
*Contact: patrick.linder@medecine.unige.ch
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RNA helicases of the DEAD-box family are involved in essentially all RNA-dependent cellular processes. In this issue of *Cell*, Sengoku et al. (2006) solve the structure of the DEAD-box protein Vasa in the presence of RNA and a nonhydrolyzable ATP analog and provide important insights into how this family of helicases unwinds RNA.

Although synthesized as a linear single-stranded molecule, RNA adopts very sophisticated secondary and tertiary structures and usually associates with proteins for its function. Chaperone proteins assist in assembling RNA into ribonucleoprotein (RNP) particles. DEAD-box proteins are the largest family of such chaperone proteins. They are present in all three domains of life and participate in essential processes such as transcription, ribosome biogenesis, splicing, RNA editing, RNA export from the nucleus, translation initiation, and RNA turnover. Prokaryotic genomes typically encode only a few such proteins, but eukaryotes have several dozen different DEAD-box proteins, many of which are essential for survival or development. All DEAD-box proteins share nine conserved amino acid sequence motifs. One of these motifs, the Asp-Glu-Ala-Asp or DEAD motif (also known as the Walker B motif), is responsible for the name of this family of proteins (Linder et al., 1989). The nine common motifs are contained within a core element, which is flanked by subfamily-specific amino- and carboxy-terminal sequences in many DEAD-box proteins. It is generally assumed that these flanking sequences interact with RNA or proteins and thereby confer specificity to these proteins (Tanner and Linder, 2001).

DEAD-box proteins have historically been considered RNA helicases because they have RNA-dependent ATPase activity and unwind RNA in an ATP-dependent manner in vitro. However, in contrast to replication-associated DNA helicases, the unwinding activity of DEAD-box helicases is not processive and is limited to short duplexes. This has led to the present view that, in vivo, DEAD-box proteins unwind only local RNA-RNA interactions of a few base pairs or dissociate proteins from an RNA, which will in turn allow other new interactions to occur. Despite their apparent nonprocessive function, previous structures of DEAD-box proteins (Benz et al., 1999, Story et al., 2001) have shown a high similarity with DNA helicases and the hepatitis C virus (HCV) NS3 RNA helicase. The core of the DEAD-box proteins consists of two RecA-like domains that contain all of the conserved motifs (Figure 1). All of the motifs are exposed to the cleft between the two domains or to the RNA binding surface. Interestingly, although individually superimposable, the relative orientations of the two domains in the various structures of DEAD-box proteins are different. This could be due to crystal packing or to the absence or presence of cocrystallizing nucleotides. Most importantly, these different orientations suggested a movement of the two subunits that could be interpreted as the action of the motor translocating along a single-stranded RNA and displacing a complementary RNA or a protein. Nevertheless, none of the structures could clearly explain the unwinding activity of DEAD-box proteins, nor could translocation be demonstrated. In particular, it was impossible to explain bidirectional unwinding, an activity that has been described for several DEAD-box proteins. Such a bidirectional activity is indeed difficult to reconcile with translocation along a single strand.

In this issue of *Cell*, Sengoku et al. (2006) present an elegant combination of structural, biochemical, and functional data on *Drosophila* Vasa. Vasa was among the first DEAD-box proteins to be identified (Linder et al., 1989). Vasa orthologs are present throughout the animal kingdom and have been linked to germline development in organisms ranging from nematodes to humans (Raz, 2000). The biological function of Vasa has been characterized in most detail in *Drosophila*, where it has been implicated in oogenesis, embryonic patterning, and germ-cell specification. Vasa is thought to carry out at least some of its functions in development by activating translation of specific mRNA targets. It binds to eukaryotic initiation factor 5B (eIF5B), a general translation factor required for recruitment of the 60S ribosomal subunit to the translational start site. Disruption of the Vasa-eIF5B interaction by site-specific mutagenesis abolishes the function of Vasa in mediating wild-type expression of Gurken pro-

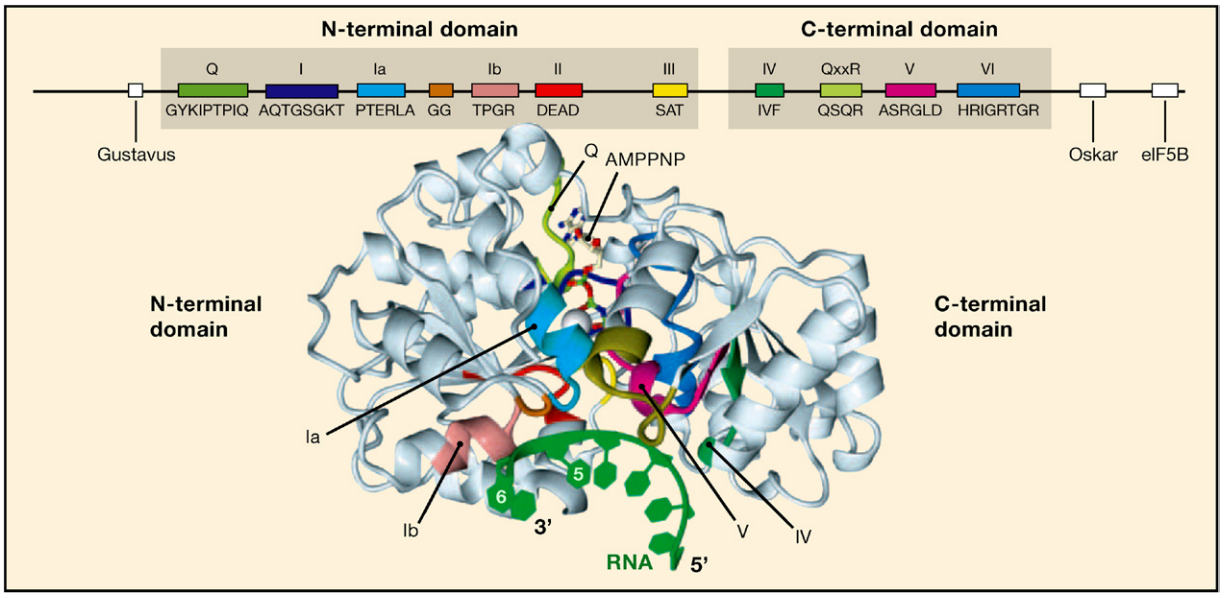

Figure 1. The DEAD-Box Helicase Vasa Bound to RNA

Nine conserved motifs are shared by all DEAD-box proteins and cluster into an N-terminal domain and a C-terminal domain. The order of these motifs is conserved throughout this family of helicases. Domains of Vasa that mediate interactions with other binding partners (such as Gustavus, Oskar, and eIF5B) lie outside of this region. The structure of the Vasa protein bound to a nonhydrolyzable ATP analog (AMPPNP) and to a single-stranded RNA substrate clearly shows the bend induced in the RNA molecule between nucleotides U5 and U6. (Structure reproduced from Figure 2B in Sengoku et al., 2006).

tein, consistent with a direct role for Vasa in activating *gurken* translation (Johnstone and Lasko, 2004). *vasa* mutations also disrupt stable accumulation of specific mRNAs in the posterior-pole plasm. Whether this phenotype involves a direct association between Vasa and these mRNAs or results from a more indirect effect remains unknown.

Sengoku et al. (2006) present the structure of a large portion of Vasa cocrystallized with an RNA and a nonhydrolyzable ATP analog and elucidate an unwinding mechanism that may be common to many DEAD-box proteins. The new and very important insight deduced from this structure is the existence of a steric clash between the bound RNA and helix α7 of domain 1. This clash induces a sharp bending of the RNA between nucleotides U5 and U6, which renders stacking interactions between U5 and U6 impossible (Figure 1). This bend is incompatible with binding of a fully double-stranded substrate, indicating a role of this bending in strand separation. Moreover, as the fragment of Vasa can unwind a blunt-ended substrate, it can bind to either strand and therefore can be considered as a bidirectional helicase, although this definition is somewhat artificial since no loading strand is required. Most interestingly, helix α7 is positioned differently in other superfamily 2 helicases, which could explain the different behavior of translocating and processive helicases such as HCV NS3 versus nonprocessive and displacing DEAD-box proteins such as Vasa. Whereas, in the translocating helicases, the energy requirement would be used for motion on the single strand, in the case of DEAD-box proteins, the energy would be required for release of the protein, which is consistent with their higher affinity for RNA in the presence of ATP.

This new structure also explains a great deal of biochemical data and agrees well with our present knowledge of the function of DEAD-box proteins. It shows that motifs Q, I, II, V, and VI are involved in ATP binding and motifs Ia, GG, Ib, IV, QxxR, and V are involved in RNA binding. The interactions with the RNA occur through the phosphates and the 2′ hydroxyl groups of the single-stranded RNA molecule. The interaction with the 2′ hydroxyl groups is consistent with the fact that DNA is generally not a substrate for DEAD-box proteins. Sengoku et al. (2006) also tested several predictions made on the basis of the structure with biochemical experiments. Strikingly, they found that mutations in motifs Ia, QxxR, V, and VI did not affect either RNA crosslinking or ATPase activity but abolished unwinding activity. Residues within these motifs interact with each other and with residues of motif III, emphasizing the importance of intraprotein interactions for the helicase activity and developmental function of Vasa, as mutations affecting interdomain interactions result in a dominant-negative phenotype.

It remains an open question why some DEAD-box proteins require single-stranded tails in the RNA substrate for unwinding to occur, whereas others, such as eIF4A, RhlE, and Vasa, do not. Results with the DEAD-box helicase eIF4A indicate that the core region alone has poor unwinding activity but can unwind

blunt-ended substrates. Perhaps, in the case of longer DEAD-box proteins, the flanking amino- and carboxy-terminal extensions direct the protein to a single-stranded overhang and increase its local concentration. This hypothesis cannot be tested in the light of the Vasa structure, as the fragment of Vasa that was crystallized lacked most of the residues outside of the core domain. However, it is in accordance with data from the bacterial DEAD-box proteins CsdA and SrmB, which need a minimum length of the single-stranded tail of the substrate for efficient activity (Bizebard et al., 2004). The absence of the flanking sequences from the structure also makes it impossible to fully model the binding sites for Vasa-specific partner proteins such as eIF5B and Gustavus (Styhler et al., 2002).

Another key issue that will require further experimentation is how substrate specificity is achieved. The particular role for Vasa in germline development suggests that it regulates only certain substrate RNAs, but the basis for this specificity is not clear from its structure, which seems to rule out sequence specificity in RNA binding. Conceivably, the flanking regions, protein cofactors, or posttranslational modifications are involved in conferring specificity to particular target RNAs. Therefore, defining the target dsRNA or RNA-protein complexes for Vasa binding remains a crucial point for further understanding of its function. Another crucial question is the regulation of DEAD-box protein activity. It is known from other DEAD-box proteins, such as yeast Ded1, which is closely related to Vasa (Cordin et al., 2004), that binding to RNA is largely stimulated by ATP binding, but binding to RNA stimulates ATP hydrolysis. Thus, to function, the DEAD-box protein needs to be directed in a timely fashion to its target RNA or kept inactive and then activated in a timely manner. How this occurs is another challenge for future experiments in the field.

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Nck Links Nephrin to Actin in Kidney Podocytes

Karl Tryggvason,[1,*] Timo Pikkarainen,[1] and Jaakko Patrakka[1]

[1]Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, 171 77 Karolinska Institutet, Stockholm, Sweden

*Contact: karl.tryggvason@ki.se

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Two papers, one in *Nature* (Jones et al., 2006) and the other in the *Journal of Clinical Investigation* (Verma et al., 2006) show that Nck adaptor proteins connect phosphorylated nephrin with actin polymerization in podocyte foot processes, structures important for slit-diaphragm formation in the kidney. Their results further our understanding of podocyte development and repair in glomerular disease.

The glomerular podocytes contribute to the kidney filter in a unique manner. They extend primary processes to the capillary surface where they form fine secondary foot processes that interdigitate with foot processes of a neighboring podocyte (Figure 1). This interdigitation results in a 40 nm wide slit between foot processes which contains a porous ultrafilter called the slit diaphragm. The foot processes have coiled actin microfilaments along their axis. The slit diaphragm proper is formed by the extracellular domains of specific transmembrane proteins, such as nephrin, the Neph proteins, and two large cadherins FAT1 and FAT2. Little is known about the extracellular interactions between the different SD proteins, but nephrin mol-